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GRANT TITLE:Genetic diversity and stability of coral-algal symbiosis

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OBJECTIVE: To characterize ecological differences between different genotypes of symbiotic algae in the corals *Montastraea annularis* and *M. faveolata*, and to assess their stability in the face of environmental change.

APPROACH:Pieces of the coral were collected from reefs in the San Blas archipelago, Panama. Our work involved sampling of *Montastraea annularis* and *M. faveolata* across a variety of depths. For each colony, two pairs of microhabitats were compared: top vs. side of column or peak, and top vs. base of colony. Zooxanthellae-enriched samples were prepared from the coral tissues. Then small-subunit rDNA was amplified by the polymerase chain reaction, and the resulting products digested with several restriction enzymes. Bands on these gels were then scored for the abundance of three major genotypes, which differ in RFLP pattern. These samples were analyzed initially for differences in distribution of the different genotypes, and subsequently compared to samples taken at adjacent sites following a coral bleaching episode associated with elevated water temperatures.

ACCOMPLISHMENTS: We were able to show that the three different genotypes have different distributions with respect to depth and position on the colony. The type C genotype is found in deeper water than the type B and A genotypes, and within a coral colony, tends to be found near the base or sides of columns/peaks. From this we may conclude that type C genotypes are distributed at locations on the reef with characteristically lower levels of light. During a bleaching event, the type C genotype is most seriously affected, and is particularly hard hit in the highest light parts of its distribution.

CONCLUSIONS: The three major genotypes of symbiotic algae found with *Montastraea* corals differ ecologically. The primary differences concern their distributions with respect to light. During unusually high temperatures, there is a negative synergy

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The productivity of coral reefs depends on a symbiosis between the coral animal and dinoflagellate algae in the genus Symbiodinium. Previous work showed that these algae are highly diverse, but the ecological distribution and significance of this diversity were not known. We characterized the symbionts of the dominant Caribbean coral Montastraea annularis sensu lato across depth and across light environments within single colonies by amplifying the small subunit rDNA gene and analysing the RFLP patterns of the products with several restriction enzymes. These studies showed that different genotypes exhibit characteristic differences in distribution, with some limited to low light environments. Shortly after doing this survey, the reefs we studied were subjected to a bleaching event in which the symbiosis between corals and algae broke down due to high temperature. By taking replicate samples adjacent to our earlier samples, we were able to show that only the low-light algae were significantly affected by the high temperatures, typically in locations near their maximum tolerable light levels. This work provided the first unambiguous documentation of how algal genotypes differ in their ecology and stability in the face of temperature stress.

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between temperature and high light, resulting in the differential loss of low-light adapted algae.

SIGNIFICANCE: This work represented the first unambiguous demonstration of ecological zonation among different genotypes of symbiotic algae. Moreover, our data provided a solution to the long standing enigma of why coral bleaching is so variable across the reef. Now that we know the ecological distributions of symbiotic algae and their vulnerability to global warming, we can begin to consider measures to enhance the long term survival of coral reefs.

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Landscape ecology of algal symbionts creates variation in episodes of coral bleaching

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Landscape ecology of algal symbionts creates variation in episodes of coral bleaching

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Reef-building corals are obligate, mutualistic symbioses of heterotrophic animals and phototrophic dinoflagellates (*Symbiodinium* spp.)[1]. Contrary to the earlier, widely accepted belief that corals harbour only one symbiont, we found that the ecologically dominant Caribbean corals *Montastraea annularis* and *M. faveolata* can act as hosts to dynamic, multi-species communities of *Symbiodinium*. Composition of these communities follows gradients of environmental irradiance, implying that physiological acclimatization[2–4] is not the only mechanism by which corals cope with environmental heterogeneity. The importance of this diversity was underlined by analysis of a natural episode of coral bleaching. Patterns of bleaching could be explained by the preferential elimination of a symbiont associated with low irradiance from the brightest parts of its distribution. Comparative analyses of symbionts before and after bleaching from the same corals supported this interpretation, and suggested that some corals were protected from bleaching by hosting an additional symbiont that is more tolerant of high irradiance and temperature. This 'natural experiment' suggests that temporal and spatial variability can favour the coexistence of diverse symbionts within a host, despite the potential for destabilizing competition among them[5,6].

The corals *Montastraea annularis* and *M. faveolata* each host three distantly related taxa[7] of the dinoflagellate genus *Symbiodinium*, denoted *A*, *B* and *C*, that are identified by restriction-fragment length polymorphisms (RFLPs) in genes encoding small ribosomal RNA (srRNA)[7]. *A* and *B* are common in shallow-water corals (high-irradiance habitats), whereas *C* predominates in deeper corals (low-irradiance habitats). Mixed samples $A + C$ and $B + C$, common at

intermediate locations[7], suggest that symbionts may actually exist as complex communities that track differences in irradiance within a colony[2].

To test this hypothesis we sampled four locations in each of 46 colonies (Fig. 1). All *M. faveolata*, and all but one colony of *M. annularis*, yielded two or three types of symbionts. As predicted, *Symbiodinium A* and *B* dominated locations with higher, downwelling irradiance (communities 1 and 2, unshaded colony tops), and *C* dominated locations of lower irradiance (communities 3 and 4, colony sides and shaded colony tops) ($P < 0.001$; χ^2 test). These patterns of intra-colony zonation largely disappear at slightly greater depths (8–11 m in *M. annularis*, and 6–12 m in *M. faveolata*), where *Symbiodinium C* is predominant overall[7]. As before[7], *Symbiodinium A* was more common in *M. faveolata* than in *M. annularis*.

Analyses at a finer scale (~1 cm) confirmed that symbionts occupy distinct but overlapping habitats (Fig. 2). Unshaded columns of *M. annularis* create a localized gradient of low (on the side, no downwelling) to high (on the top, full downwelling) irradiance, which we sampled along transects. At intermediate depths (3–7 m) this gradient coincides with the transition from *Symbiodinium C* to *B*, *B* + *C*, or *A* (Fig. 2a–d). Analyses of shallower (1–2 m) and deeper (9–12 m) corals (Fig. 2d) show that depth[7] and intracolony zonation of the symbionts occur in parallel. These consistent patterns strongly argue that zonation is controlled by ambient irradiance. Furthermore, experimentally toppled columns, which experienced immediate and severe changes in irradiance gradients, largely re-established expected patterns of symbiont zonation during a six-month period (Fig. 2e,f). This response shows that the patterns are maintained dynamically.

Symbioses between corals and dinoflagellates are stable mutualisms, with the notable exception of coral bleaching, which involves the loss of symbionts and/or photosynthetic pigments[3,8]. Bleaching is an ecologically important but poorly understood response to environmental stress[3,8,9]. Many bleaching events exhibit intraspecific variation distributed within and among habitats in ways

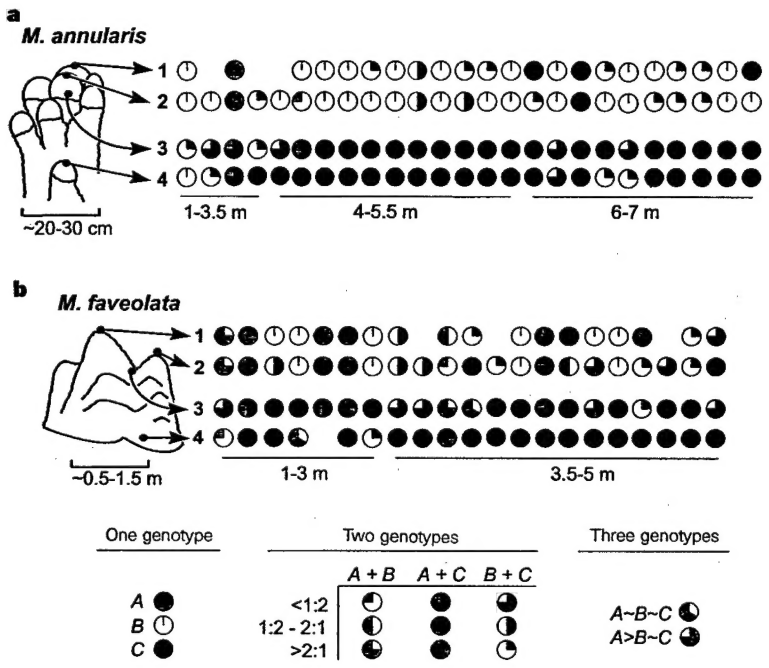

Figure 1 Symbiont communities in *M. annularis* (**a**) and *M. faveolata* (**b**). Each symbol represents one core sample that contained *Symbiodinium A*, *B*, *C* or mixtures of taxa summarized according to the code shown below. Columns in the data matrices represent individual coral colonies (depth increases from left to right), and rows represent locations of higher (rows 1 and 2) and lower (rows 3 and 4) irradiance, as defined in the diagrams to the left. Samples were collected in January 1995.

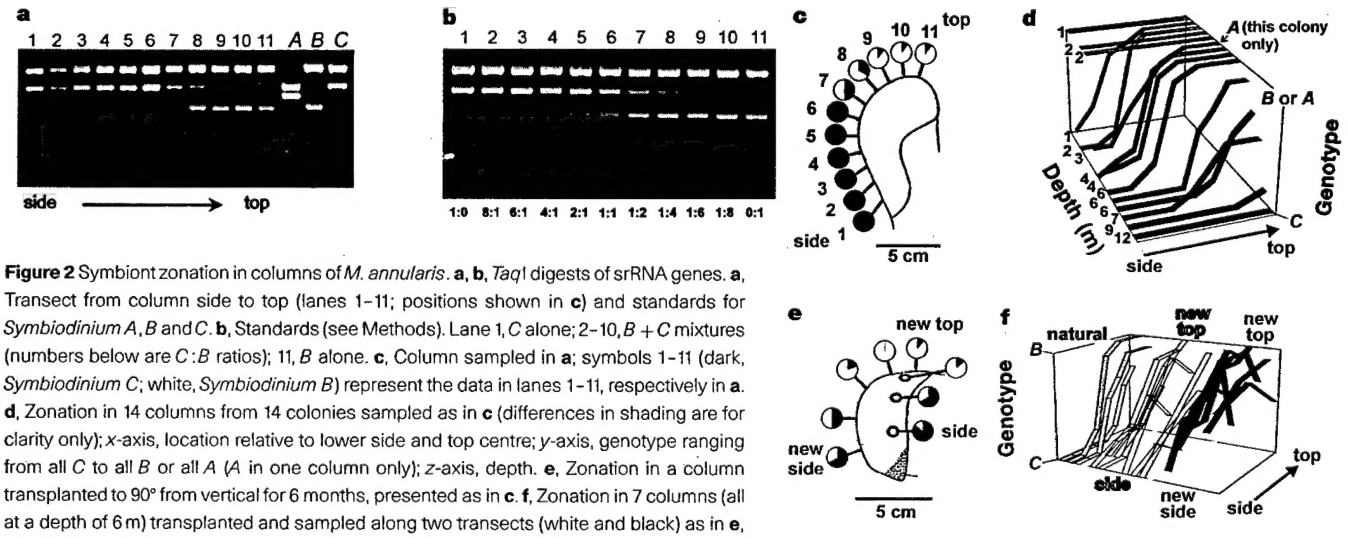

Figure 2 Symbiont zonation in columns of *M. annularis*. **a**, **b**, *Taq*I digests of srRNA genes. **a**, Transect from column side to top (lanes 1–11; positions shown in **c**) and standards for *Symbiodinium A*, *B* and *C*. **b**, Standards (see Methods). Lane 1, *C* alone; 2–10, *B* + *C* mixtures (numbers below are *C*:*B* ratios); 11, *B* alone. **c**, Column sampled in **a**; symbols 1–11 (dark, *Symbiodinium C*; white, *Symbiodinium B*) represent the data in lanes 1–11, respectively in **a**. **d**, Zonation in 14 columns from 14 colonies sampled as in **c** (differences in shading are for clarity only); *x*-axis, location relative to lower side and top centre; *y*-axis, genotype ranging from all *C* to all *B* or all *A* (*A* in one column only); *z*-axis, depth. **e**, Zonation in a column transplanted to 90° from vertical for 6 months, presented as in **c**. **f**, Zonation in 7 columns (all at a depth of 6 m) transplanted and sampled along two transects (white and black) as in **e**, presented as in **d**; zonation in natural columns at a depth of 6 m (from **d**) are plotted for comparison (grey).

that are difficult to explain[9–12]. Because irradiance and temperature act synergistically to induce bleaching[12–14], and the symbionts of *M. annularis* and *M. faveolata* exhibit different associations with irradiance (Figs 1 and 2), we hypothesized that symbiont polymorphism underlies this variation.

We observed 'paling' in several colonies of *M. annularis* and *M. faveolata* on 18 September 1995, and bleaching was extensive by the second week in October, both in Panama and elsewhere[15]. At our study site, this event was 'typical': like a similar event there in 1983 (ref. 16), it followed a prolonged excursion above the mean summer maximum of temperature (Fig. 3e); it also coincided with atypically high water clarity (data in ref. 17), which implies increased irradiance at depth[2]. We also observed complex[9,10,18] bleaching patterns in both *M. annularis* and *M. faveolata*. Bleaching was rare or slight at both shallow (<2 m) and deep (>15 m) sites; in between, however, both species displayed a curious pattern, with shallower colonies bleached preferentially in shaded places (Fig. 3a,b) and deeper colonies bleached preferentially in unshaded places (Fig. 3c,d). Among *M. annularis* partitioned as in Fig. 1a (communities 1 and 2 versus 3 and 4) and by depth (above 8 m versus below 8 m), this difference was significant ($n = 76$ colonies, 64 bleached; $P < 0.05$, χ^2 test). Some colonies exhibited a 'ring' of bleaching at the boundary between column top and side (Fig. 3a). *M. faveolata* colonies are not easily partitioned into two distinct irradiance microhabitats, but they clearly showed the same reciprocal pattern (Fig. 3b,d), with a shallower (~6 m) centre. Such observations have previously been hard to explain[12] because the environment is isothermal, and the associations with irradiance and colony morphology are inconsistent.

Symbiont zonation provides a simple hypothesis to explain these bleaching patterns. Bleaching was disproportionately common in what seems to be the upper limit of *Symbiodinium C*'s 'adaptive zone': low-irradiance parts of corals in shallower water, and high-irradiance parts of corals in deeper water. Slight increases in temperature and irradiance might push these symbioses, but not others, beyond some physiological limit, resulting in bleaching. This hypothesis accounts for our bleaching observations, including areas of slight bleaching[9,15,19] (see Fig. 3a, b), if *Symbiodinium C* were expelled selectively from mixed symbiont communities.

An analysis of symbionts collected in late October supported this interpretation of events. Post-bleaching samples were obtained <1 cm from many sites sampled the previous January (Fig. 1). All available samples from communities that had previously contained mixtures of *Symbiodinium C* plus either *A* or *B* (or both) were identified (Fig. 1) and analysed. We reasoned *a priori* (Fig. 2) that these sites accurately defined the limit of *Symbiodinium C* in corals under non-bleaching conditions. Such mixtures also allow the fates of different symbionts to be compared directly. We also tested archived samples taken at the same time as, and <1 cm away from, the original (pre-bleaching event) samples. In every case, *Symbiodinium* srRNA RFLPs in these replicate, pre-bleaching pairs were equivalent (data not shown), indicating that the small distance between pre- and post-bleaching samples was unlikely to be significant.

As predicted, *Symbiodinium C* had decreased in relative abundance in all 18 communities tested (see Fig. 4a–c). Absolute responses of different symbionts within a mixed community were compared by using estimates of relative abundances (from RFLP data; see Fig. 4a–c) to partition direct counts of symbionts into each genotype (Fig. 4d). Losses of *Symbiodinium C* were typically close to 100%, whereas *B* underwent a median decrease of 14%, and *A* more than doubled in 3 of 5 instances. The single sample that contained all three symbionts exhibited these same trends (Fig. 4c, d). Changes in colony chlorophyll contents and subjective assessments of bleaching paralleled changes in symbiont numbers (Fig. 4e). From these data we can tentatively rank the 'fitness' of the different *Symbiodinium* taxa as symbionts under 'bleaching conditions'. The ranking obtained in this manner is: $A > B \gg C$.

Our study provides a fuller understanding of *M. annularis* and *M. faveolata*, which are dominant members of western Atlantic reefs[20]

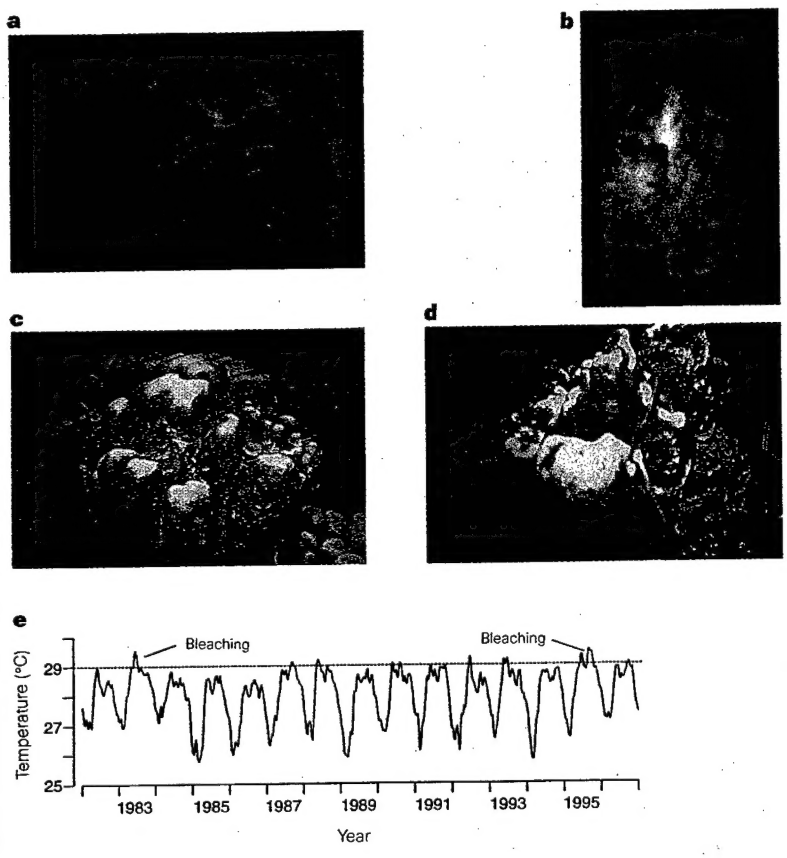

Figure 3 Bleaching in *M. annularis* (**a**, **c**) and *M. faveolata* (**b**, **d**) at the study site on 28 October 1995 showing 'shallow' (**a**, **b**) and 'deep' (**c**, **d**) patterns. **e**, Sea surface temperatures (three-week running means, from satellite data[30]) at the San Blas Islands, Panama. Temperatures above 29 °C in 1983 and 1995 were associated with coral bleaching[16] (this study). Records from our study site (at 7-m depth) since 1993 (Marine Environmental Sciences Program, Smithsonian Tropical Research Institute) corroborate satellite data.

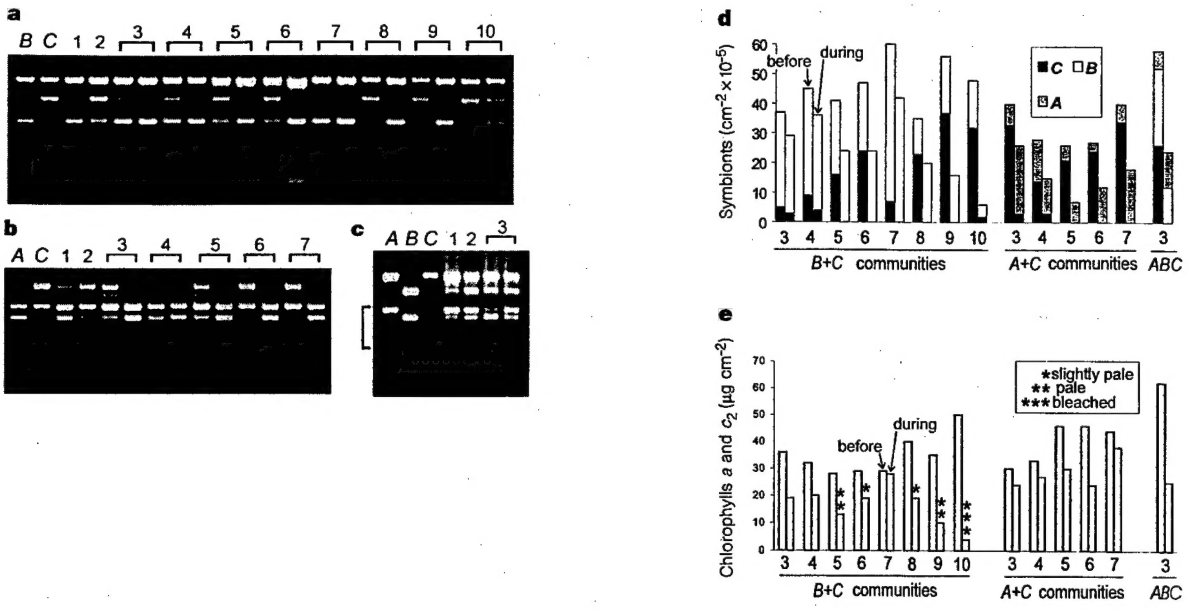

Figure 4 Symbiont communities before (January 1995) and during (October 1995) an episode of coral bleaching. **a**–**c**, Lanes contain *Taq*I (**a**, **b**) or *Dpn*II (**c**) digests of srRNA genes. **a**, Standards for *B*, *C* and *B* : *C* ratios of 8 : 1 (lane 1) and 1 : 1 (lane 2); lane pairs compare symbionts before (left) and during (right) bleaching in *M. annularis* (lanes 3–6) and *M. faveolata* (lanes 7–10). **b**, Standards for *A*, *C* and *A* : *C* ratios of 2 : 1 (lane 1) and 1 : 8 (lane 2); lane pairs 3 (*M. annularis*) and 4–7 (*M. faveolata*) compare symbionts as in **a**. **c**, Standards for *A*, *B*, *C* and equal amounts of *A*, *B* and *C* (lane 1) and *A* and *B* (lane 2); lane pair 3 compares symbionts in *M. faveolata*, as in **a** and **b**. The vertical bracket identifies bands that identify each symbiont. **d**, Densities of *A* (grey), *B* (white) and *C* (black) before and during bleaching (left and right bars of each pair, respectively) in samples reported in **a** (*B* + *C*, communities 3–10), **b** (*A* + *C*, communities 3–7) and **c** (*ABC*, community 3). **e**, Chlorophyll contents of the samples reported above, presented as in **d**. Samples were scored as 'normal' (not marked) or 'slightly pale', 'pale', or 'bleached' (marked by asterisks) when collected.

and are widely used as model systems in reef biology and geology[11,13,18,19,21,22]. Each coral 'species' encompasses one animal and dynamic, multi-species communities of symbiotic dinoflagellates. This strongly contradicts the 'one host, one symbiont' view of reef corals[1], in which host taxa alone are adequate units of biodiversity, environmental variability is accommodated largely by physiological acclimatization[2–4], and bleaching variability is often not understood[12]. We conclude that polymorphic symbiont communities underlie the broad distributions[20] and bleaching ecology of these corals. Directed shifts in symbiont populations following extreme environmental change (Figs 2e, f and 4) suggest that similar shifts may also occur over annual cycles of environmental variation[19]. For these corals, and for mutualisms in general, the ability to cope with environmental change through changes in symbiont community composition reflects the selective advantage of hosting several distinct symbionts, despite the potential for destabilizing competition among them[5,6].

How typical and important are the patterns documented here? *M. annularis* and *M. faveolata* in the Bahamas also host *Symbiodinium A*, *B* and *C* (data not presented), and published photographs[18] and descriptions[9,10] of bleaching elsewhere strongly resemble our own (Fig. 3a–d). With respect to Caribbean corals in general, bleaching is often predominant at intermediate depths[9]. We can attribute this pattern (and its within-colony correlate) in *M. annularis* and *M. faveolata* to symbiont polymorphism and zonation. Moreover, at least three other species of Caribbean corals host (at least) both *Symbiodinium A* and *C* (ref. 23; our unpublished data). For other species, which might host multiple but not so distantly related symbionts, refinements of *Symbiondinium* taxonomy would be essential. However, symbiont polymorphism does not exclude the significance of other attributes that are important features of coral biology, such as physiological acclimatization of hosts and symbionts[2–4] and genetic differences among hosts[11,14].

It has been hypothesized that a global warming trend, with increased frequencies of world wide coral bleaching induced by increasing temperature or ultraviolet irradiation, could have catastrophic consequences for many living coral reefs[3,8,21]. Alternatively, coral communities may adjust to climate change by recombining their existing host and symbiont genetic diversities[24–26]. Our findings supply a precedent for this idea: that one species of coral can flexibly host more than one taxon of *Symbiodinium* to produce symbioses with distinct ecological properties. For example, *M. annularis* and *M. faveolata* might adjust to a warmer Atlantic ocean by hosting more *Symbiodinium A* and less *Symbiodinium B* and *C*. However, long-term consequences of such replacements would depend on how they affect rates of coral growth and reproduction. □

Methods

Field collections and manipulations. Coral samples were collected at Aguadargana reef in San Blas, Panama[27] by coring (1.1 cm^2 surface area) and freezing immediately in liquid nitrogen (data in Figs 1 and 4). Other colonies (data in Fig. 2) were sampled by removing a defined circular area (~0.12 cm^2) of living tissue from freshly collected colonies with an airbrush. In transplant experiments (Fig. 2e, f), columns of *M. annularis* were broken off ~15 cm below the living tissue, turned on their sides, and cemented (at the non-living base) back to the colony at a comparable location; this increased (new top), decreased (new side), or did not change (side) local irradiance. All 28 transplants at a depth of 6 m seemed to be normal after 6 months. Analyses of non-transplanted (control) columns showed that natural zonation patterns were stable over this period (data not shown).

Laboratory analyses. Symbionts and symbiont DNA were isolated from frozen[7] and from fresh[28] samples. Nuclear srRNA genes were amplified using the 'universal' PCR primers ss5 and ss3 (all data in Fig. 1) or a combination of ss5 and the '*Symbiodinium*-biased' primer ss3Z (all data in Figs 2 and 4), and analysed with *Taq*I and *Dpn*II (data were consistent in every case)[7]. The biased primer (ss3Z) does not discriminate (within this study) against unknown, specific *Symbiodinium* genotypes (discussed in ref. 28), as confirmed by sequencing[7] and by comparing results from 'universal' and 'biased' amplifications of 30 samples that contained two genotypes (*A* + *C* or *B* + *C*) in various

proportions.

Cloned srRNA genes from *Symbiodinium A*, *B* and *C* were used as standards[7]. They were amplified singly and from defined mixtures of two (Fig. 2b) or three (Fig. 4c) types[7] to assign field samples to classes of symbiont relative abundance by visual comparison (Fig. 2a, b). To validate this procedure, approximately equal numbers of *A*, *B* and *C* cells, from three natural isolates of each type, were mixed in pairwise combinations and analysed. The results implied that *Symbiodinium B* and *C* yield (on a per-cell basis) equal signals, whereas *A* yields about twice that amount. Standard mixtures of cloned genes were adjusted accordingly.

Symbiont densities and chlorophyll contents (Fig. 4d, e) were determined from haemacytometer counts (8 replicate grids per sample) and spectrophotometrically from methanol extracts[29], respectively. These symbionts were isolated quickly (with minimal washing) from frozen samples at 4 °C under dim light. Symbiont genotypes, numbers and chlorophyll contents were obtained from subsamples of each isolate.

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